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Original article

Pharmacological properties and preliminary phytochemical analysis of *Pseudocaryopteris foetida* (D.Don) P.D. Cantino leaves

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ABSTRACT

Medicinal plants have significant contribution in pharmaceutical industries being producers of compounds utilized as precursors for drug development. A plant of Lamiaceae family; *Pseudocaryopteris foetida* had not been investigated for its biomedical potential. Current research was aimed to investigate phytochemical analysis, cytotoxic potential and antioxidant activity of crude methanolic extract and fractions of *Pseudocaryopteris foetida* (leaves). The preliminary phytochemical analysis of crude methanolic extracts and fractions of *Pseudocaryopteris foetida* revealed that plant is rich in phenolic and flavonoid classes of secondary metabolites while presence of tannin was observed only in crude methanolic extract. The cytotoxicity was determined using brine shrimp lethality test. Different concentrations (25, 50, 100, 150, 200 and 250 μg/mL) of crude methanolic extract and fractions exhibited dose dependent cytotoxicity. However, The LD_{50} for all the extracts was more than 200 μg/mL indicating weak cytotoxic potential of *Pseudocaryopteris foetida.* The antioxidant capabilities of crude methanolic extract and fraction of *Pseudocaryopteris foetida* were analyzed by in vitro bio assays including DPPH, ABTS, Reducing power and phosphomolybdate antioxidant assays using ascorbic acid as standard. The crude methanolic extract showed IC_{50} (256.38 ± 0.6 and 314.95 ± 1.1 μg/mL) for DPPH and ABTS respectively, while total antioxidant capacity was calculated as 55.79 ± 0.5 μg/mL for crude methanolic extract of *Pseudocaryopteris foetida* while ascorbic acid indicated total antioxidant capacity of 71.89 ± 2.3 μg/mL. Study concluded that leaves of *Pseudocaryopteris foetida* were the rich source of antioxidant phytochemicals. Based on preliminary investigations further research should be focused to isolate bioactive phytochemicals as leading source of clinical medicines in future.

1. Introduction

From early times human have intimate relationship with plants, as the plants and their products are fully integrated into human lives and smoothly running the need wheel. Active ingredients of plants are able to cure many chronic and acute diseases (Adnan et al. 2015). Secondary metabolites of plants like alkaloids, flavonoids, tannins and phenolics are potent phytochemicals that directly affect human metabolism (Sher et al. 2014). The phytochemical analysis of plants presents an idea about the chemical nature of compounds that confer host plant with certain effective biological activity. The family "Lamiaceae" is the 6th largest plant family of flowering plants (Li et al. 2016) with 6000–7000 species distributed in 236–240 genera (Badamtsetseg et al. 2012; Khaled-Khodja et al. 2014; Rehan et al. 2014; Carovic-Stanko et al. 2016). Plants of Lamiaceae family are rich in secondary metabolites like phenolics, flavonoids, alkaloids etc and hence well known for their therapeutic value. For instance *Mentha spicata* is used to cure

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gastric, intestinal, cold and muscular problem, in traditional folk medicine system. Presence of phenolics in the leaves of *Mentha spicata* can be linked to anti-oxidant potential of plant (Soni and Sosa, 2013). Similarly, *Thymus daenensis, Thymus vulgaris* and *Zataria multiflora* are used to cure stomach problems, muscular pain, cough and inflammation (Saderi and Abbasi, 2011). *Zataria multiflora* has also been reported for anti-oxidant, anti-microbial and digestive stimulant properties (Ingole, 2016).

Genus *Pseudocaryopteris* belongs to family Lamiaceae and comprised of three species namely *P. bicolor, P. foetida* and *P. paniculata*. *Pseudocaryopteris* genus was first described in 1998 (Cantino et al. 1998). *Pseudocaryopteris foetida* (D.Don) P.D. Cantino is an evergreen shrub with limited distribution. It is native to Pakistan, Northern India and Nepal (Govaerts, 2003). According to Global Biodiversity Information Facility (GBIF), *Pseudocaryopteris foetida* was also reported from Mexico between 20.9 °N and 103.8 °W at an elevation of 1000 m from sea level (Li et al. 2016). *Pseudocaryopteris foetida* had not been evaluated for its pharmacological potential to the best of our knowledge. Based on the phytochemical profile of members from Lamiaceae family, it is assumed that *Pseudocaryopteris foetida* might contain potent phytochemicals having wide range of bioactivities. So the present study was designed to explore *Pseudocaryopteris foetida* leaves for preliminary qualitative and quantitative phytochemical analysis. Hence crude methanol extract of leaves and polarity based fractions were analyzed for their antioxidant and cytotoxic properties.

2. Experimental

2.1. Collection of plant material and preparation of crude extract

Leaves of *Pseudocaryopteris foetida* were collected from New Murree (Union Council Bann, Tehsil Murree, Pakistan) during February 2018. The plant was identified by an expert taxonomist at Department of Botany, PMAS-Arid Agriculture University Rawalpindi, Pakistan. The voucher specimen was deposited in herbarium of Quaid-i-Azam University Islamabad, Pakistan for future reference. Fresh leaves were washed, shade dried and ground to powder form. Then, the powdered leaves (30 g) were dipped in 300 mL methanol for extract preparation. The crude methanol extract was filtered and concentrated using rotary evaporator. Crude extract was stored at 4 °C until further used.

2.2. Fractionation of crude extract

Crude methanol extract of *Pseudocaryopteris foetida* leaves was fractionated into n-Hexan, ethyl acetate and methanol fractions using separating funnel. The fractions were also stored at 4 °C until further used.

2.3. Phytochemical analysis

2.3.1. Qualitative analysis

Presence or absence of certain phytochemicals was confirmed by practicing standard protocols with slight modification. Cardiac glycosides, flavonoids, steroids, phlobatannins, saponins and tannins were tested (Soni and Sosa, 2013). Presence or absence of phenols, proteins, anthraquinone and carbohydrates were tested following procedure adopted by Geetha and Geetha (2014). Alkaloids and coumarins were detected by the methods as described by Labiad et al. (2017).

2.3.2. Quantitative analysis

To quantify phenolic constituents of leaves of *Pseudocaryopteris foetida* and fractions, Follin-Ciocalteu reagent method was used as describe by Singleton and Rossi (1965). Gallic acid was used as standard and spectrophotometer (Cecil, 7000 series) analysis was carried out at λ 760 nm. For the determination of total flavonoid contents of each extract, the standard aluminum chloride method was used as suggested by Zhishen et al. (1999) with slight modifications. Quercetin was used as standard and spectrophotometer analysis was carried out at λ 415 nm.

2.4. Evaluation of cytotoxic properties Pseudocaryopteris foetida leaves

The cytotoxic potential of crude extract and fraction of *Pseudocaryopteris foetida* leaves was assessed by brine shrimp lethality test. The stock solutions were prepared by dissolving crude extract and fractions in DMSO. To achieve maximum tolerable concentration<1.25% DMSO (v/v) was used to prepare each dilution (Geetha and Geetha, 2014). A total six concentrations (dilutions) 1000, 750, 500, 250, 100 and 50 μg/mL of crude extract and fractions were prepared in sea water. Vincristine sulfate, Etoposide and $K_2Cr_2O_7$ were used as control. The percent (%) lethality of the brine shrimp nauplii was measured and calculated by using Abott's formula.

$$Pt = [(Po - Pc)/(100 - Pc)] \times 100$$

Whereas, Po = Observed mortality and Pc = Control mortality.

Median lethal concentration (LC_{50}) was analyzed by the linear regression method in which percentage (%) mortality against correspondent log of concentration was plotted (Dai and Mumper, 2010).

2.5. Antioxidant potential of Pseudocaryopteris foetida leaves

The antioxidant potential of crude extract and fraction of *Pseudocaryopteris foetida* leaves were analyzed using DPPH method, ABTS radical scavenging assay, reducing power assay and Phosphomolybdate assay.

2.5.1. DPPH assay

The antioxidant potential of crude extracts and fractions was evaluated as described by Ishaque et al. (2018). Ascorbic acid was used as a standard, while methanol was used as blank. A stock solution (5 mg/mL) of each extract was made in methanol and subsequent dilutions of 25, 50, 100, 150, 200 and 250 μg/mL were prepared. Then, 200 μL from each dilution was mixed with 200 mL of DPPH solution and placed in dark at 25–30 °C for 30 min. Next, the contents of each reaction tube were analyzed using spectrophotometer at a wavelength of 517 nm, and free radical quenching potential was determined using the following equation:

$$Scavenging\,(\%) = [Absorbance\,(_{control}) - -Absorbance\,(_{sample})/Absorbance\,(_{control})] \times 100$$

2.5.2. ABTS radical scavenging assay

An equal volume of the stock solution of ABTS (mM) and potassium persulphate ($K_2S_2O_8$) was kept in darkness for 12–16 hrs at room temperature. Before assay, dilution of ABTS solution was made in ethanol to yield an absorbance value of 0.700 ± 0.02 at 734 nm. Plant extracts were mixed with various concentrations of the resultant solution (2 mL), reaction mixture was vortexed and after 30 min absorbance was recorded at 734 nm. The similar treatment was repeated with various concentrations (650–50 μg/mL) of the standard ascorbic acid. Following formula was applied to determine the radical scavenging activity (Labiad et al. 2017).

$$RSA\,(\%) = [Absorbance\,(_{control}) - -Absorbance\,(_{test})/Absorbance\,(_{control})] \times 100$$

2.5.3. Reducing power assay

The procedure employed by Peteros and Uy (2010) was used to figure out the reducing power of extracts. Five concentrations of

each extract were taken and mixed with phosphate buffer and Potassium ferricyanide (2 mL each). Mixture was incubated for 20 min at 50 °C and later 10% of trichloroacetic acid (2.5 mL) was added to the mixture. Mixture was centrifuged for 10–15 min at 3000 rpm and upper layer of centrifuged mixture (3 mL) was mixed with distilled H_2O (2 mL) and 0.1% $FeCl_3$ (0.5 mL). This solution was then preceded for recording absorbance at 700 nm. Extended absorbance value of reaction mixture indicated enhanced reducing power. Ascorbic acid was used as positive control.

2.5.4. Total antioxidant capacity of extract (Phosphomolybdate assay)

Phosphomolybdate method was applied to ascertain the capacity of the crude extract and fractions for antioxidants. Reagent solution (1 mL) comprised of 28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate was mixed with small fraction (0.5 mL) of test solution. Tubes were covered and incubated for 90 min in water bath at 95 °C. Later, test samples were cooled and the absorbance was measured at 765 nm against a blank reagent. Reagent solution (1 mL) and the appropriate volume of solvent incubated under the similar conditions were used as blank. Ascorbic acid was utilized as standard. Given formula was used to calculate antioxidant capacity value:

$$Antioxidant\ effect\,(\%) = [Absorbance\ (_{control}) - -Absorbance\ (_{sample})/Absorbance\ (_{control})] \times 100$$

2.6. Statistical analysis

All experiments were performed in triplicate. Results were analyzed statistically by using 10 analytical software USA, and Microsoft Excel software. IC_{50} values were calculated by standard linear regression curve.

3. Results

3.1. Phytochemical analysis

The crude methanolic extract (CME) of *Pseudocaryopteris foetida* leaves showed strong positive results for presence of phenolics, tannins, terpenoids and flavonoids. While, the fractions including n-hexane fraction (nHF), ethyl-acetate fraction (EAF) and methanol fraction (MF) showed strong positive results only for phenolics and flavonoids. The results of qualitative analysis of *Pseudocaryopteris foetida* leaves and its fractions are given in Table 1. The quantitative analysis showed that CME of *Pseudocaryopteris foetida* leaves were rich in phenolics (163.5 ± 1.2 mg/g GAE), flavonoids (37.54 ± 0.7 mg/g QE) and tannin (17.5 ± 1.7 mg/g TAE) as presented in Table 2.

3.2. Evaluation of cytotoxic properties Pseudocaryopteris foetida leaves

The crude extract of *Pseudocaryopteris foetida* leaves and polarity based fractions were subjected to brine shrimp lethality test (BSLT) to analyze their cytotoxic properties and results were compared with standard cytotoxic compounds i.e. $K_2Cr_2O_7$, Vincristine and Etoposide. The mean percentage lethality caused by various concentrations of test solutions against brine shrimps is presented in Fig. 1. Results indicated dose dependent cytotoxic effects as the concentration was raised from 50 to 1000 μg/mL. The results of cytotoxic activity were comparable with standard cytotoxic compounds with maximum percentage lethality of 100% when treated with CME, NHF, $K_2Cr_2O_7$, Vincristine and Etoposide at a concentration of 1000 μg/mL while EAF and MF indicated 95 and 90% lethality at the same concentration. On the other hand ME and CME indicated LD_{50} at a concentration of 211 and 217.11 μg/mL respectively while $K_2Cr_2O_7$, Vincristine and Etoposide indicated LD_{50} at a concentration of 149.33, 167.83 and 168.23 μg/mL respectively (Fig. 2).

3.3. Evaluation of antioxidant potential of Pseudocaryopteris foetida leaves

Free radical scavenging potential of *Pseudocaryopteris foetida* leaves crude extract and fractions were assessed by different antioxidant assays included DPPH, ABTS, Reducing power assay and Phosphomolybdate antioxidant assay. The percentage free rad-

Table 1
Classes of phytochemical present in leaves extracts of *Pseudocaryopteris foetida*.

Classes of Phytochemicals	Crude Methanol Extract (CME)	Methanol fraction (MF)	Ethyl-acetate fraction (EAF)	n-hexane fraction (nHF)
Phenols	++	++	++	+
Tannins	++	–	–	+
Saponins	+	+	–	–
Resins	+	–	+	–
Terpenoids	++	+	+	++
Steroids	+	+	+	+
Alkaloids	+	+	+	+
Phlobatannins	–	–	–	–
Flavonoids	++	++	++	+
Carbohydrates	++	++	++	+
Proteins	++	++	++	+
Coumarins	+	+	+	+

++ = Strong presence, + = presence of phytochemicals, - = absence of phytochemicals. n = 3.

Table 2
Quantitative analysis of total phenolic, total flavonoid and total tannic acid contents in leaf extracts of *Pseudocaryopteris foetida*.

	Crude extract	Methanol fraction	Ethyl acetate fraction	n-hexane fraction
Total phenolic contents (mg/g GAE[♦])	163.5 ± 1.2	128.45 ± 1.2	107.94 ± 0.3	90.49 ± 0.3
Total flavonoid contents (mg/g QE[†])	37.54 ± 0.7	24.8 ± 1.2	21.36 ± 1.7	16.05 ± 1.7
Total Tannins contents (mg/g TAE[⁂])	17.5 ± 1.7	*	*	*

[♦] = Gallic acid equivalent,
[†] = Quercetin equivalent,
[⁂] = Tannic acid equivalent,
* = Not Observed. n = 3.

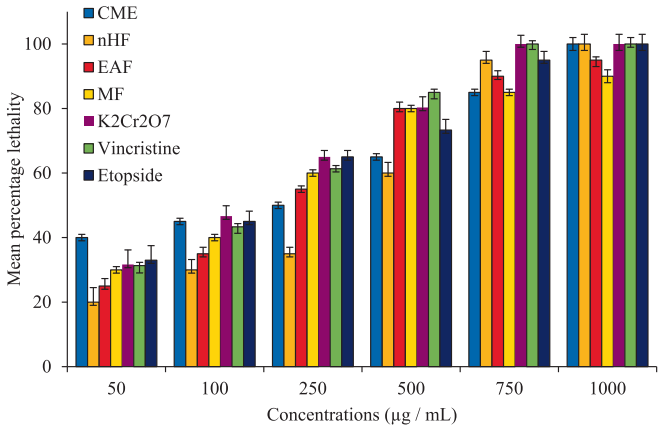

Fig. 1. Effect of various concentrations of *Pseudocaryopteris foetida* leaves fractions and standard drugs against Brine shrimps after 24 h of exposure. All the values were ± S.D of triplicate ($p \leq 0.05$). CME = Crude methanolic extract, nHF = n-Hexan fraction, EAF = Ethyl acetate fraction and MF = Methanol fraction.

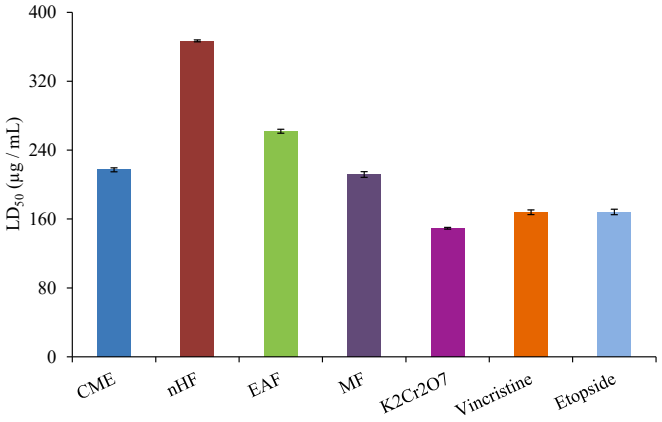

Fig. 2. Comparison between LD_{50} values of *Pseudocaryopteris foetida* extracts / fractions and known cytotoxic drugs. All the values were ± S.D of triplicate ($p \leq 0.05$). CME = Crude methanolic extract, nHF = n-Hexan fraction, EAF = Ethyl acetate fraction and MF = Methanol fraction.

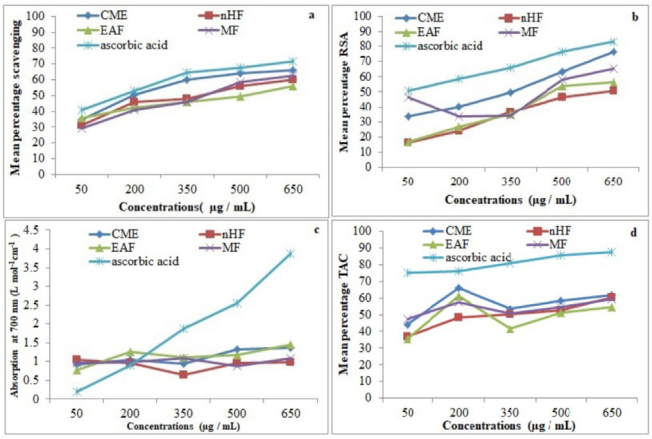

Fig. 3. Assessment of antioxidant potential of crude extract and fractions of *Pseudocaryopteris foetida.* a) DPPH free radical scavenging assay, b) Radical scavenging potential by ABTS assay, c) Reducing power assay and d) Determination of total antioxidant capacity by Phosphomolybdate assay. Ascorbic acid was used as standard. n = 3, $p \leq 0.05$. CME = Crude methanolic extract, nHF = n-Hexan fraction, EAF = Ethyl acetate fraction and MF = Methanol fraction.

ical scavenging potential of test solutions and standard at various concentrations is presented in Fig. 3 (a, b and d). The results of reducing power assay are presented in Fig. 3c. The absorption of light directly corresponded to the free radical scavenging potential of test solutions at given concentration (Fig. 3c). Different antioxidant assays showed different IC_{50} values (Fig. 4). The IC_{50} of CME of *Pseudocaryopteris foetida* leaves were 256.38 ± 1.2, 314.95 ± 0.7 and 55.79 ± 0.7 µg / mL for DPPH, ABTS and TAC respectively.

4. Discussion

Plants ranked second in medicinal uses, according to WHO more than 80% of healthcare needs are fulfilled by plants that are used to cure chronic and acute diseases (Aziz et al. 2018). Species from family Lamiaceae are known for ornamental value. They can also be utilized as spices, vegetable, cosmetic products and medicines. These species have been traditionally used for food preservation, flavoring and medicinal purposes, due to both their preventive and curative properties. Species of Lamiaceae family have complex mixture of bioactive compounds with potential anticancer, anti-inflammatory, antibacterial, antioxidant, antimicrobial and antiviral activities (Carovic-Stanko et al. 2016). *Pseudocaryopteris* genus of Lamiaceae family was introduced in 1998 by Cantino and it is comprised of three plant species (Cantino et al. 1998). In the current study preliminary phytochemical constituents of *Pseudocaryopteris foetida* along with cytotoxic and antioxidant potential of this plant species were determined. The preliminary qualitative phytochemical analysis revealed the presence of valuable different classes of phytochemicals in leaves of *Pseudocaryopteris foetida* including phenols, tannins, saponins, resins, terpenoids, steroids, alkaloids, flavonoids, cardiac glycosides, carbohydrates and proteins. The presence these diverse categories of bioactive compounds in *Pseudocaryopteris foetida* indicated the medicinal potential of *Pseudocaryopteris foetida* as these bioactive constituents are multifunctional natural products acting through multiple targets by being anti-inflammatory, and antioxidant (Begashaw et al. 2017; Baloch et al. 2019). The quantitative studies revealed that the leaves of *Pseudocaryopteris foetida* were rich in phenolics, flavonoids and tannins. Similarly, the polarity based fractions were rich in phenolics and flavonoids while fractions lack tannin. Absence of tannins from fractions can be justified due to their instability in other solvents. Some solvents can be recommended as good or optimal solvents for extraction due to their capacity in conserving and stabilizing the chemical structure of the targeted compounds (Do et al., 2014, Monton and Luprasong, 2019).

The cytotoxic potential of crude extract and fractions indicated elevation with increase in concentration of extracts. Similar observations were also reported by Ogbole et al. (2017) while working with medicinal plants from Nigeria ethnomedicine. Similarly previous studies indicated that the cytotoxicity of the plant extracts can be linked with the presence of bioactive phyto-constituents like tannins, flavonoids and triterpenoid (Aliomrani et al. 2017; Elansary et al. 2018; Ganame et al. 2021; Ishaque et al. 2021). Results of LD_{50} analysis indicated that crude extract and fractions of *Pseudocaryopteris foetida* are bioactive as Standard brine shrimp lethality assay defines that an LD_{50} value < 1000 µg/mL is considered bioactive in toxicity evaluation of plant extracts (Kabubiia et al. 2015). The MF showed least LD_{50} (211.70 ± 3.3 µg/mL) indicating highest cytotoxic potential followed by CME (217.17 ± 2.3 µg/mL). Similar observations were also reported by Nascimento et al. (2009) while working with extracts of *Mentha arvensis*. Previous studies reported that extracts with LD_{50} < 500 µg/mL are considered moderate cytotoxic while extracts exhibiting LD_{50} < 100 µg/mL are strongly cytotoxic (Nguta et al., 2012; Osamudiamen et al. 2020). So the leaves of *Pseudocaryopteris*

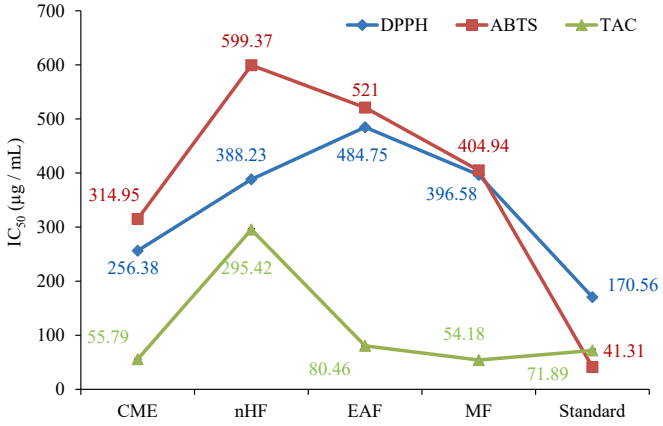

Fig. 4. Comparison of free radical scavenging potential of *Pseudocaryopteris foetida* leaves in terms of IC_{50} by DPPH, ABTS and Phosphomolybdate (TAC) assay. n = 3, $p = \leq 0.05$. CME = Crude methanolic extract, nHF = n-Hexan fraction, EAF = Ethyl acetate fraction and MF = Methanol fraction.

foetida have moderate cytotoxic potential with varying cytotoxicity in various fractions that is MF > CME > EAF > nHF.

Antioxidant activity of plant extracts can be quantified using different methods and it is recommended to use at least two methods for determination of antioxidant activity of plant extracts for reliable results as these methods differ in sensitivity (Milan et al. 2010; Gupta et al. 2017). In current study four methods were used to determine antioxidant potential of plant. The DPPH free radical scavenging assay being most famous has been used for investigating antioxidant properties of multiple food and nonfood components (Ul-Haq et al. 2012). We observed a direct relationship between dose and radical quenching potential in all samples using DPPH as previously reported Mohan et al. (2019). As a result of ABTS radical scavenging assay the MF of *Pseudocaryopteris foetida* showed strong free radical scavenging percentage (46.77%) at 50 µg/mL but at higher concentrations it was decreased. The absorbance of MF at 50 and 200 µg/mL were higher than ascorbic acid indicating the presence of phytochemicals with better antioxidant potential than ascorbic acid. Similar finding is reported while determination of antioxidant potential of epicarp and mesocarp of *Lagenaria siceraria* fruit (Ahmed et al. 2014). The CME of *Pseudocaryopteris foetida* at 650 µg/mL, showed 61.5% TAC as compared to ascorbic acid (87.38%) at the same concentration. Thus each fractionated sample (n-hexane, methanol and ethyl acetate) possessed considerable TAC ranging between 61.51 and 54.47% at 650 µg/mL.

The IC_{50} of CME and fractions of *Pseudocaryopteris foetida* were compared with standard antioxidant compound (ascorbic acid). The order of IC_{50} for DPPH were EAF > MF > nHF > CME > ascorbic acid while for ABTS and TAC bioassays; nHF > EAF > MF > CME > a scorbic acid and nHF > EAF > MF > ascorbic acid > CME respectively. The results suggested that antioxidant activity quantified by DPPH method was higher with IC_{50} value of 170.56 µg/mL as compared to ABTS with IC_{50} of 314.95 µg/mL. These findings are in agreement to previously published data regarding woody species of arid zones of Mexico, where values of antioxidant activity quantified by DPPH were above those obtained by ABTS (Wong-Paz et al. 2015).

5. Conclusion

In conclusion, *Pseudocaryopteris foetida* leaves were found to be rich in flavonoids and phenolics while alkaloids, tannins and coumarins were also identified from various fractions. Owing to the presence of these bioactive phytochemicals, plant extracts exhibited significant antioxidant and moderate cytotoxic activity. As preliminary screening of extracts and their pharmacological potential can lead towards discovering new drug candidates, therefore, further extensive research like isolation of the phyto-constituents and studies on clinical level is recommended to develop novel therapeutic formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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